

The University of Chicago

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TRANSLOCATION OF AMMONIUM
2,4-DICHLOROPHENOXYACETATE
BY BEAN PLANTS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY

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Reprinted from
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ABSORPTION AND TRANSLOCATION OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE BY BEAN PLANTS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 597

ELROY L. RICE

Introduction

Several investigators (2, 8, 12) have reported that the addition of Carbowax 1500 to 2,4-dichlorophenoxyacetic acid (2,4-D) markedly increased the responses made to the growth substance when applied to certain plants. With other plants, however, applications of wholly aqueous solutions are just as effective (2). One purpose of the present experiments was to shed some light on the reasons for the increased effectiveness of ammonium 2,4-dichlorophenoxyacetate [$\text{NH}_4(2,4\text{-D})$] when applied to the Red Kidney bean in a solution containing Carbowax 1500. In the present investigation, absorption of $\text{NH}_4(2,4\text{-D})$ by the Red Kidney bean leaf was studied by direct measurement.²

Temperature has been shown (3, 4, 5, 9) to have a marked effect on the herbicidal activity of 2,4-D and related compounds. No detailed studies, however, have been made of the influences of temperature on absorption and translocation of such materials.

Considerable work has been done on the relation of light intensity to absorption and translocation of 2,4-D and related substances (7, 14). It has been reported (7, 14) that local reaction of a treated bean leaf occurs in the dark, the

leaf tip curling back when the growth-regulator was placed near the tip. These investigators were unable to determine how much 2,4-D was absorbed in the dark, because they had no method of measuring absorption directly. Consequently, another objective of the present experiments was to compare the absorption of $\text{NH}_4(2,4\text{-D})$ by the Red Kidney bean leaf under various light intensities.

MITCHELL and BROWN (7) concluded that no translocation of 2,4-D occurred in the dark in the snap bean. They stated, also, that no stem curvature, which was used as a criterion of translocation, resulted during 1 day following application of 2,4-D to snap beans kept in a shaded location (250-500 foot-candles at noon, on a clear day). WEAVER and DEROSE (14) found that kidney-bean plants treated with 2,4-D and kept in the shade did not exhibit as much curvature 1 day after treatment as those treated in the same manner in full sunlight. Despite the close agreement of results of studies on translocation under various light intensities, it seemed desirable to include such a study in the present investigation in conjunction with experiments on absorption.

Material and methods

The Red Kidney bean was chosen as the test plant because its reactions to 2,4-D and related compounds are fairly well known and because of its adaptability to greenhouse conditions and its rapid rate of growth.

All seeds were hand picked to insure

¹ This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

² The term "absorption" is used in this paper to mean simply the entrance of the ammonium salt into the leaf tissues, irrespective of the forces which are operative or the degree of penetration and later translocation.

uniformity of size and freedom from injury. They were planted in soil in 4-inch glazed pots, three seeds per pot. Before treatment, one plant in each pot was removed, leaving the two most nearly uniform plants. For each of the six experiments 150 pots were planted, but only a maximum of 120 pots with the most nearly uniform plants were used. This procedure insured having all the plants for each experiment very uniform in height and in area of primary leaves. All the plants were grown in the same greenhouse room and were moved to the various test rooms at the desired time before treating with the growth-regulator.

Three test rooms were used for study of the effects of temperature and Carbowax on absorption and translocation. The lowest temperature range (46° – 58° F.) was maintained in a refrigerated room with thermostatic control. The two higher ranges of temperature were maintained within fairly constant limits in rooms in which temperature values did not change rapidly. Basement rooms, all with the same temperature range, were used for the experiments at various light intensities.

Each test room (except the dark room) was equipped with a bank of twelve "daylight" fluorescent tubes which could be raised or lowered so as to regulate the light intensity at the surfaces of the primary leaves to the desired amount. A daily photoperiod of 13 hours was employed while the plants were in the test rooms (except in the dark room).

In all series in the light the plants were treated 2 hours after the lights came on by applying, with a 0.2-ml. Kahn pipette, 0.05 ml. of water containing a known amount of $\text{NH}_4(2,4\text{-D})$ (with or without 0.5% Carbowax 1500 in different series) to the base of the midrib on the upper surface of one primary leaf. The amount

so applied could be controlled consistently within approximately 1 μg . The aqueous solutions of $\text{NH}_4(2,4\text{-D})$ were made by dissolving either 340 mg. or 670 mg. of the salt (the amount depending on whether the treatment was to consist of 34 μg . or 67 μg .) in distilled water and making up to 500 ml. To make the aqueous solution containing 0.5% by weight of Carbowax 1500, 670 mg. of the salt were dissolved in 2.5 gm. of melted Carbowax. After hardening, this was dissolved in distilled water and made up to 500 ml. in a volumetric flask, so that 0.05 ml. would contain 67 μg . of the growth-regulator. One of the primary leaves was treated before much expansion of the first trifoliate leaf had occurred, since preliminary experiments had shown that the responsiveness of kidney-bean plants to a given amount of $\text{NH}_4(2,4\text{-D})$, in terms of both stem curvature and growth inhibition, decreased rapidly with increase in age. Similar results on the effect of age have been reported (16) for several other species.

The plants in the dark room were treated in a similar manner with the aid of a photographic developing lamp with an orange filter. It was found that this light did not allow the formation of chlorophyll in *Avena* coleoptiles in a 24-hour period of exposure. It is probable that such a light had little, if any, effect on metabolic processes in the bean plants during the very few minutes required for application of the solution or during the very few minutes required to clip the treated leaves.

The treated leaves were cut off at the bases of the petioles at various intervals after application (4, 10, 24, 48, and 72 hours), so as to interrupt further translocation of the growth-regulator from the treated leaf to other parts of the plant. Fourteen treated leaves were clipped at

each interval in each series. Nine days after application of $\text{NH}_4(2,4\text{-D})$ to the primary leaves, the fresh weights of the expanded trifoliate leaf blades (including petiolules but not petioles) on treated and control plants were obtained. These weights were used in evaluating the relative amounts of growth inhibition which resulted for the various time intervals during which translocation was possible. The amount of such growth inhibition in similar experiments (14) has been assumed to be proportional to the amount of growth-regulator translocated from the treated leaf into the stem.

If a small amount of a growth-regulator, such as $\text{NH}_4(2,4\text{-D})$, is applied to one side of the stem of a succulent plant, such as bean, cell enlargement is accelerated on the treated side, and stem curvature results (7). Therefore, any stem curvature which follows the application of such a growth-regulator to a leaf blade is an indication that a stimulus has been translocated from the treated leaf to the region of the stem in which the curvature occurs. Some experimenters (2) have used the amount of stem curvature as a measure of the relative effectiveness of various treatments with growth-regulators. In the present experiments the stem curvatures were measured, at the time of clipping the primary leaves, with a transparent plastic protractor.

The clipped primary leaves were placed on moist filter paper in moist chambers and were immediately subjected to the following procedure. Each leaf was placed in a 100-ml. beaker, and the small treated area was washed thoroughly with 20 ml. of distilled water blown forcibly from a pipette. This water was poured into a 25-ml. volumetric flask, and the beaker was rinsed with about 4 ml. of distilled water which was added to the flask. The contents of the

flask were made up to volume with distilled water.

The same general procedure was used in handling the clipped leaves from the untreated control plants. When Carbowax was used in the treatment, an equal amount was also applied in 0.5% aqueous solution to one primary leaf of each of the control plants at the time of treating the other plants in the series.

In the determination of the amount of $\text{NH}_4(2,4\text{-D})$ left on the surface of a treated leaf at the time of clipping, the solution resulting from the washing of the treated area was tested with a Beckman Quartz Spectrophotometer, model DU, according to a slight modification of the method developed by BANDURSKI (1). All density readings were taken at 2300 Å, using 5-cm. silica sample cells. Control blanks, consisting of the washings from control plants, were used in all cases. The density of each blank was checked against that of distilled water. It was found that the density readings of most were similar. However, the density value of an occasional blank would be above or below the usual value, possibly because of some dirt or soluble phenolic compounds washed from the surface of the control leaves. Consequently, it was decided to correct all density readings on the basis of a common blank. The slight variation in the density readings of the blanks indicates that the values of the density readings of the treated-leaf washings may have been affected by the presence of compounds other than $\text{NH}_4(2,4\text{-D})$ in a few instances. However, the actual error, in terms of micrograms of $\text{NH}_4(2,4\text{-D})$ recovered from the leaf, was very small and would tend to average out for any one series in each experiment.

By experimentation the proper combination of treatment, volume of test so-

lution, and sample-cell size was determined, so that most of the density readings on the spectrophotometer would fall within the optimum range of the instrument.

After the last group of treated leaves had been clipped in each series—72 hours after application of $\text{NH}_4(2,4\text{-D})$ —all the plants were removed to a greenhouse room and were allowed to remain until the ninth day after application of the growth-regulator. At this time the fresh weights of the trifoliate leaf blades were obtained. In experiment F these weights were taken on the eleventh day after application.

Results

EXPERIMENT A.—The purpose was to study the effects of temperature on the absorption and translocation of $\text{NH}_4(2,4\text{-D})$ applied in aqueous solution.

The plants were placed in the various test rooms 48 hours before being treated. One room was kept at the temperature range of $46^{\circ}\text{--}58^{\circ}\text{F}$. (series 1), one at $79^{\circ}\text{--}82^{\circ}\text{F}$. (series 2), and the other at $86^{\circ}\text{--}92^{\circ}\text{F}$. (series 3). A primary leaf on each plant was treated, according to the method already described, with $67\text{ }\mu\text{g}$. of the salt. At the time of application the first trifoliate leaf was just starting to expand.

In series 2 and 3 the maximum amount of $\text{NH}_4(2,4\text{-D})$ had entered the leaf within approximately the first 4 hours after application (fig. 1). Maximum absorption in the lowest temperature range (series 1) had occurred at the end of 48 hours or perhaps slightly less.

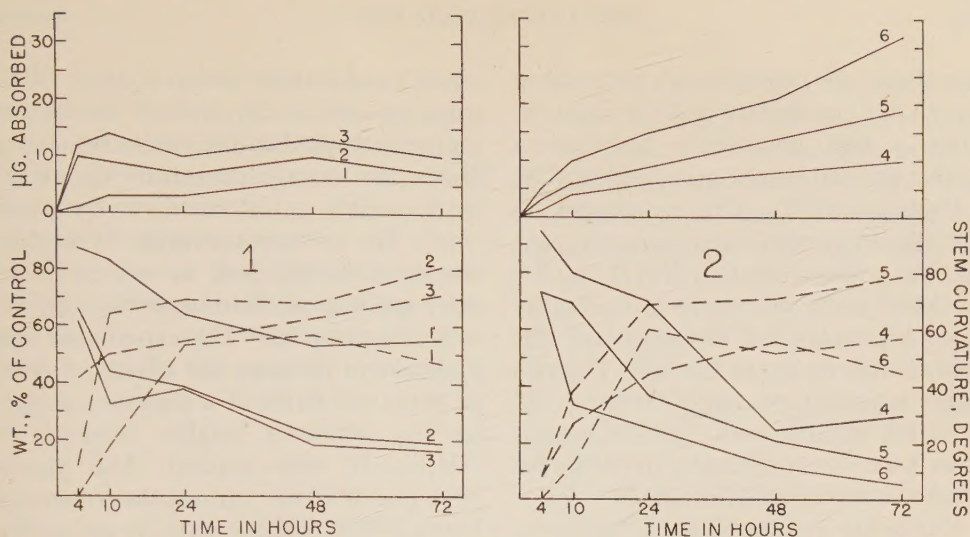
The amount of $\text{NH}_4(2,4\text{-D})$ apparently absorbed was measured as the difference between the known amount applied and the amount washed off the primary leaf. Experimental errors, especially in determining the latter amount, would

help to account for some of the fluctuations in the curves showing apparent absorption in series 2 and 3 (fig. 1). In addition, it will be recalled that each point in a curve is an average of the amounts of $\text{NH}_4(2,4\text{-D})$ apparently absorbed by fourteen plants at the specified time, each group of fourteen consisting of different plants. Moreover, it was found in some cases that individual plants, although outwardly similar to others, showed quite pronounced differences in the amount of the salt apparently absorbed. In spite of these possibilities of experimental error, however, the trends of the curves are definite, and the differences in their values at any one time are for the most part statistically significant (table 1).

The amount of the salt which apparently entered the leaf was greater at the higher temperatures (fig. 1). Table 1 shows the differences between the mean amounts absorbed in the three series for each clipping and, also, for all clippings considered collectively.

Regardless of temperature, the treated primary leaves had to remain attached over 24 hours to allow enough absorption and translocation of the salt to result in approximately maximum inhibition of growth, as measured by the fresh weights of the harvested trifoliate leaf blades (fig. 1).

No stem curvature was found in any plant within 4 hours after application of the salt in series 1, but practically every treated plant in the two higher temperature ranges showed some stem curvature during this period. At the end of this first 4-hour period, the average amount of curvature was greatest at the highest temperature (series 3). However, when the treated leaves were left attached for over 4 hours, the plants in series 2 showed the greatest average stem curva-



FIGS. 1-2.—Effects of temperature and Carbowax 1500 on: (above) absorption of $\text{NH}_4(2,4\text{-D})$ by Red Kidney bean leaves; (below) stem curvature (broken line), and fresh weights of harvested trifoliate leaf blades of treated plants expressed as a percentage of control averages (solid line). Each plant treated at base of midrib of one primary leaf with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution. In series 4, 5, and 6 (fig. 2) solution also contained 0.5% by weight of Carbowax 1500. Abscissa represents time of removal of treated primary leaves after application of growth substance. Light: 900 foot-candles; 13-hour daily photoperiod. Temperature ranges: in series 1, 2, and 3 (fig. 1)—46°–58°, 79°–82°, and 86°–92° F., respectively; in series 4, 5, and 6 (fig. 2)—46°–58°, 76°–80°, and 86°–94° F.

TABLE 1
EFFECT OF TEMPERATURE ON ABSORPTION OF $\text{NH}_4(2,4\text{-D})$, WITH OR WITHOUT CARBOWAX 1500. DATA BASED ON FOURTEEN PLANTS AT EACH TEMPERATURE RANGE AND EACH CLIPPING. ALL VALUES SHOWN ARE POSITIVE

Series 1, 46°–58° F. Series 4, 46°–58° F.
Series 2, 79°–82° F. Series 5, 76°–80° F.
Series 3, 86°–92° F. Series 6, 86°–94° F.

	DIFFERENCE BETWEEN SERIES IN $\mu\text{G.}$ ABSORBED (NEAREST WHOLE VALUE)					
	Time of clipping after treatment					All clippings
	4 hours	10 hours	24 hours	48 hours	72 hours	
	NH ₄ (2,4-D) in water only					
Series 2—series 1.....	9*	6*	4*	4†	1	5*
Series 3—series 2.....	2	5	3	3	3†	3*
	NH ₄ (2,4-D) in water containing 0.5% Carbowax 1500					
Series 5—series 4.....	2*	2*	3†	6*	9*	5*
Series 6—series 5.....	2	4*	6*	7*	13*	6*

* Significant at 0.01 level.

† Significant at 0.02 or 0.05 level.

ture at any one time through the 72-hour period, despite the fact that the plants in series 3 had apparently absorbed a greater amount of the growth-regulator.

EXPERIMENT B.—This experiment essentially duplicated experiment A except that the aqueous solution of $\text{NH}_4(2,4\text{-D})$ contained 0.5% by weight of Carbowax 1500. A primary leaf of each plant was treated with 67 μg . of the salt. The two higher temperature ranges (series 5 and 6) varied slightly from those in experiment A but were sufficiently like them for a direct comparison of results.

Unlike the results without Carbowax 1500, absorption of $\text{NH}_4(2,4\text{-D})$ continued to take place during the entire 72-hour period in all three series (fig. 2). The total amount absorbed in 72 hours was practically double the amount absorbed at the equivalent temperature range without Carbowax, although absorption during the first 4 hours was slower with Carbowax in the higher temperature ranges.

Again, as with the wholly aqueous treatment, the total amount of the salt absorbed was positively correlated with temperature (fig. 2, table 1).

As in experiment A, the treated primary leaves had to remain attached more than 24 hours in all series to permit sufficient absorption and translocation of the salt to result in approximately maximum inhibition of growth, as measured by the fresh weights of the harvested trifoliate leaf blades (fig. 2).

Likewise as in experiment A, stem curvature did not occur in any plant in the lowest temperature range (series 4) within 4 hours after application (fig. 2). Most treated plants in series 5 and 6 showed some curvature during this period, although the average curvature was not quite so great as in experiment A. The average curvatures were the same in

series 5 and 6 at the end of 4 hours. In all cases in which the treated leaves remained attached to the plants for over 4 hours, the average curvature was greatest in series 5 (intermediate temperature). The average curvature in series 6 was even slightly less, 24 and 72 hours after application, than in series 4 (fig. 2).

EXPERIMENT C.—This experiment was designed to measure the effects of temperature on plants of a slightly younger age to which a smaller amount of $\text{NH}_4(2,4\text{-D})$ was applied. The plants were placed in the various test rooms 48 hours before treating with 34 μg . of the salt in aqueous solution. At the time of application the first trifoliate leaf had not yet started to expand. The temperature ranges in series 7, 8, and 9 were 46°–58° F., 68°–72° F., and 78°–82° F., respectively.

The amount of the salt absorbed was not measured. The fresh weights of the trifoliate leaf blades were inversely correlated with the temperature values maintained during the period of treatment, as in experiments A and B (fig. 3). Again, no stem curvature occurred within 4 hours after application in any plants treated in the lowest temperature range (series 7). In series 8 and 9, however, stem curvature resulted in every plant during this period, and the degree of mean curvature at the end of 4 hours was positively correlated with temperature as in experiments A and B.

EXPERIMENT D.—Half the plants (series 10) were placed in light of 900 foot-candles with a 13-hour daily photoperiod and half (series 11) in darkness, 24 hours before application of $\text{NH}_4(2,4\text{-D})$. The temperature range in both series was 76°–82° F. Each plant was treated with 67 μg . of the salt in water only. At the time of application the first trifoliate leaf had not yet started to expand.

The amount of the salt apparently absorbed in the dark was significantly greater than in the light (fig. 4, table 2). Much of the absorption took place during the first 4-hour period after application, as in experiment A.

There was no stem curvature in the plants treated in the dark, and the average weight of their trifoliolate leaf blades was essentially the same as the average

86° F. At the time of application the first trifoliolate leaf was just starting to expand.

There was a continued absorption of the salt in the dark during the entire time that the material remained on the leaf (fig. 4), as was true when Carbowax was

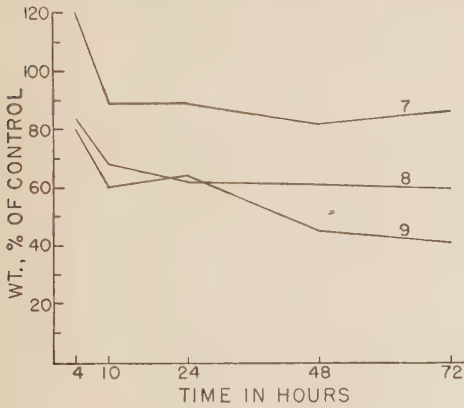


FIG. 3.—Relative growth inhibitions of kidney-bean plants treated, on one primary leaf, with 34 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution, as shown by fresh weights of harvested trifoliolate leaf blades (weights expressed as percentages of control averages). Temperature ranges of series 7, 8, and 9—46°–58°, 68°–72°, and 78°–82° F., respectively. Light: 900 foot-candles; 13-hour daily photoperiod. Abscissa as in figs. 1–2.

of the corresponding controls (fig. 4). The primary leaves treated in the dark folded upward along the midrib and became wavy and crinkled. The average weight of the trifoliolate leaf blades of plants treated in the light was inversely correlated with duration of treatment, as in all other experiments.

EXPERIMENT E.—The plants (series 12) were placed in the dark 48 hours before each was treated with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in water with 0.5% Carbowax 1500 and were left in the dark for the following 72 hours. The temperature of the dark room varied from 82° to

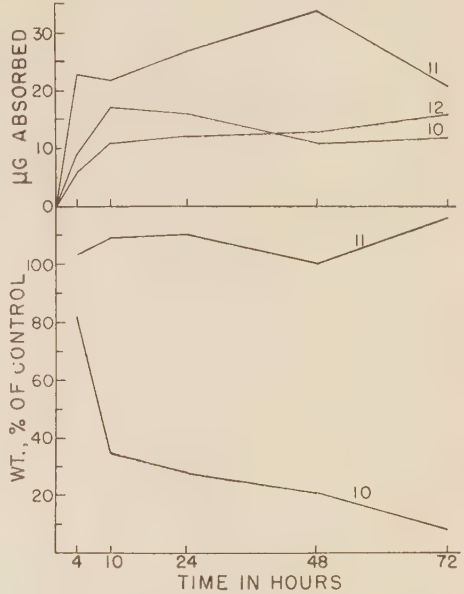


FIG. 4.—Effect of light on: (above) absorption of $\text{NH}_4(2,4\text{-D})$ by bean leaves; (below) stem curvature (broken line) and growth inhibition (solid line), expressed as in figs. 1–2, of plants treated at base of midrib of one primary leaf with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution. Series 12, 0.5% Carbowax 1500 added to solution. Light: series 10, 900 foot-candles with 13-hour daily photoperiod; series 11 and 12, complete darkness. Temperature ranges: series 10 and 11, 76°–82° F.; series 12, 82°–86° F. Abscissa as in figs. 1–2.

used in the treatment in the light in experiment B. There was no stem curvature, but, as in experiment D, the treated primary leaves folded upward along the midrib and became crinkled and wavy. Growth of the trifoliolate leaf blades was not visibly affected by the treatment.

EXPERIMENT F.—Series 13, of eighty plants, was placed in light of 100 foot-candles, series 14 in 900 foot-candles (13-hour photoperiod in both series), and

series 15 in complete darkness, all 24 hours before each plant was treated with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in water only. The temperature range for all series was the same, 69°–77° F. The first trifoliate leaf had not yet started to expand at the time of application.

back at the tips or folded upward along the midrib. They usually became slightly wavy and crinkled also.

The treated primary leaves on plants in light of 100 foot-candles became wavy and crinkled but not to the same degree as those in the dark. There was some

TABLE 2
EFFECT OF LIGHT ON ABSORPTION OF WHOLLY AQUEOUS SOLUTION OF $\text{NH}_4(2,4\text{-D})$
DATA BASED ON FOURTEEN PLANTS FOR EACH SERIES AT EACH CLIPPING

Series 10, 900 foot-candles light	Series 13, 100 foot-candles
Series 11, complete darkness	Series 14, 900 foot-candles
Series 15, complete darkness	

DIFFERENCE BETWEEN SERIES IN $\mu\text{G.}$ ABSORBED (NEAREST WHOLE VALUE)						
Time of clipping after treatment					All clippings	
4 hours	10 hours	24 hours	48 hours	72 hours		
Experiment D—Temperature 76°–82° F						
Series 11—series 10.	+14†	+5	+11†	+23*	+9†	+12*
Experiment F—Temperature 69°–77° F.						
Series 14—series 13.	+ 1	+1	+ 2	— 1	+1	+ 1
Series 15—series 14.	+ 3	+6*	+ 4†	+ 7*	+5*	+ 5*
Series 15—series 13.	+ 4†	+7*	+ 6*	+ 6*	+6*	+ 6*

* Significant at 0.01 level.

† Significant at 0.02 or 0.05 level.

The amounts absorbed at 900 foot-candles and at 100 foot-candles were essentially the same. There were no statistically significant differences between the amounts absorbed by these series at any one time or when all clippings were considered together (table 2).

More of the salt was absorbed in the dark than in either of the series in the light (fig. 5). Most of these differences were statistically significant (table 2).

There was no stem curvature in series 15. The treated primary leaves on these plants in the dark, however, either curled

stem curvature in most of the treated plants in this series, but the average curvature was less at every measurement than for plants at 900 foot-candles (fig. 5). The average curvature in plants at 100 foot-candles was considerably greater 10 hours after treatment than at any other time.

The weight of the harvested trifoliate leaf blades of the plants treated in the dark approximated that of the corresponding controls (fig. 5). This value for plants treated at 100 foot-candles (series 13) was considerably less than the cor-

responding controls in those cases in which the treated leaves remained attached for over 24 hours. The average weight of trifoliate leaf blades (expressed as a percentage of the average of controls) in series 14 was considerably less, however, than in series 13.

In all three series in this experiment, as in all other experiments in which Carbowax was not used, most of the absorption of the salt apparently took place within the first 4-hour period after application.

Discussion

When $\text{NH}_4(2,4\text{-D})$ was applied in water without Carbowax 1500, in most cases the water was observed to evaporate completely, resulting in crystallization of the salt still on the surface, within approximately 4 hours after application in the higher temperature ranges. In these cases it was found that most of the absorption of the salt occurred during this period (figs. 1, 4, 5). In the lowest temperature range the treated area remained moist for a considerably longer period of time, and absorption continued beyond the first 24-hour period after application (fig. 1). These results of measurement of the absorption of the salt in aqueous solution agree fairly closely with the conclusions reached indirectly by WEAVER and DEROSE (14), who, in interpreting the results of WEAVER *et al.* (15), stated that "almost maximum damage ensued if young plants [Red Kidney bean] were not subjected to an artificial rainfall until 6 hours after exposure [to aqueous $\text{NH}_4(2,4\text{-D})$ sprayed on them], indicating maximum entry of the compound into the plant within this period." By "maximum entry" the authors apparently meant the maximum under the conditions of the experiment and not the total amount that could enter the plants under any circumstances.

In all experiments except experiment F, the plants used grew in the greenhouse during warm weather. When they were treated, the drop of solution remained at the base of the midrib of the treated leaf. The plants used in experiment F grew in the greenhouse during cool weather and required several days longer than the others to reach the same apparent stage of development. When their leaves were

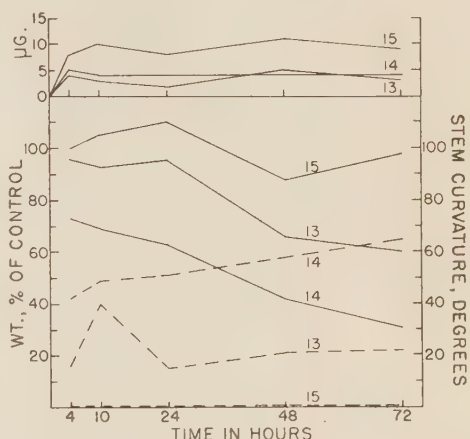


FIG. 5.—As in fig. 4. Plants treated with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in wholly aqueous solution. Temperature range: 69°–77° F. Light intensities for series 13, 14, and 15: 100 foot-candles, 900 foot-candles, and complete darkness, respectively. Daily photoperiod of 13 hours for series 13 and 14.

treated with $\text{NH}_4(2,4\text{-D})$ in water (no Carbowax), the solution tended to creep along the midrib and thence out along the lateral veins. As a consequence, drying occurred quite rapidly, and the amount of the salt absorbed was considerably less than by plants of the same apparent stage of development similarly treated in experiment D. Whether the behavior of the drop was an important factor in the difference in absorption can only be conjectured. SMITH (11), using wholly aqueous solutions of $\text{NH}_4(2,4\text{-D})$ in herbicidal studies, found that sprays of relatively large-droplet size (561–250 μ av. diam.) were more effective than

those of smaller-droplet size ($30\ \mu$ av. diam.). A possible interpretation of his results would suggest that the length of time required for evaporation of the water applied in treatment is an important factor influencing the total amount of the salt absorbed when no hygroscopic agent is added. It should be pointed out that the temperature range in experiment D was somewhat higher than in experiment F. This might account for some of the greater absorption in experiment D. The amount absorbed in experiment F, however, was even less than that absorbed in the lowest temperature range of experiment A by plants in a more advanced stage of development. These observations on the behavior of the drop suggest that there may be differences in the leaf surfaces of plants grown in cool and warm weather which may condition their absorption of aqueous sprays.

In contrast to the limited absorption of the salt after the first 4 hours when applied in wholly aqueous solution are the results obtained when Carbowax 1500 was added to the solution (Figs. 2, 4). At every temperature range tested and in complete darkness as well as in light, the presence of Carbowax 1500 in the solution resulted in the salt being absorbed continuously for 72 hours. A suggestion made by ENNIS and BOYD (2) thus seems to be the correct explanation of the action of Carbowax in increasing the effectiveness of various growth substances applied to the kidney bean. They stated that "the presence of Carbowax effects the retention of moisture on the leaf surface for an interval sufficient to permit absorption of the compound by the epidermal leaf tissues."

It was found that $\text{NH}_4(2,4\text{-D})$ in aqueous solution entered the kidney-bean leaf in the lowest temperature tested ($46^\circ\text{--}58^\circ\text{F.}$), although the rate of entry at low

temperatures was considerably less than at higher temperatures. However, with Carbowax 1500 added, the amount of salt absorbed in 72 hours in the lowest temperature range was as great as in the intermediate temperature range without Carbowax.

In experiments A and B, the total amount of salt absorbed was positively correlated with temperature. It is impossible to say whether the differences in the rates of absorption at various temperatures resulted from the effects of temperature on diffusion phenomena and permeability or whether there were structural differences of the leaves themselves which affected the diffusion of the salt into them. The latter seems doubtful, since the temperature differential was maintained for only 48 hours before application.

No stem curvature in response to treatment was exhibited by any plant in the lowest temperature range ($46^\circ\text{--}58^\circ\text{F.}$) within 4 hours after application, while practically every plant in the intermediate and highest temperature ranges in all experiments showed some curvature within that period. The lack of visible response at the lowest temperature could be a consequence of the effect of temperature on the rates of one or all the following processes: absorption of $\text{NH}_4(2,4\text{-D})$, translocation of the growth-regulator to the stem, or growth responses in the stem. It has been conclusively shown that the rate of absorption was considerably less in the lowest temperature range, so that factor could have been responsible, in part at least, for the lack of stem curvature at the end of 4 hours after application. This does not prove, however, that the other two factors were not important. Another fact which tends to complicate the interpretation of the results of the translocation

studies in low temperatures is that the treated primary leaves had to remain attached for approximately the same length of time in all temperature ranges for maximum growth inhibition (for the given conditions) to ensue. Moreover, no curling or folding of the treated primary leaves occurred in the low temperature range at any time after treatment. This was in marked contrast to the visible responses of primary leaves treated in the dark or in low light intensity and from which complete translocation of the salt apparently did not take place. The last two facts may possibly indicate that most of the growth-regulator which entered the leaf in the low temperature range was translocated into the remainder of the plant just as it was in the higher temperature ranges. However, the evidence is insufficient to determine the effect of temperature on the rate of translocation.

In the present experiments it was found that more $\text{NH}_4(2,4\text{-D})$ actually entered the leaf in the dark than in the light, the differences being highly significant in most cases. Despite this fact, no stem curvature occurred in any plant kept in the dark during the period between application and clipping of the treated leaf. Moreover, the average weight of the harvested trifoliate leaf blades of plants treated in the dark was essentially the same as that of the corresponding controls. These facts suggest either that no translocation of the growth-regulator out of the leaf occurred in the plants kept in the dark or that, even if translocated, it was not effective in evoking responses in the remainder of the plant. Two factors which may have contributed to the greater absorption by the plants in the dark were their greater succulence and the slightly longer time re-

quired for the water applied to them in treatment to evaporate.

Although the amount of the salt absorbed was essentially the same at 100 foot-candles of light as at 900 foot-candles, the average stem curvature was less at every measurement in the former series. The primary leaves treated at the lower intensity tended to become wavy, similar to those in the dark but in lesser degree, while the primary leaves treated at 900 foot-candles were not visibly affected. Moreover, the average weight of the trifoliate leaf blades of plants treated at 100 foot-candles (expressed as a percentage of corresponding controls) was more in every case than those treated at 900 foot-candles. These facts suggest that the limiting factor in the systemic activity of the salt in low light intensities was its slower translocation out of the treated leaf into the stem.

Although relative inhibition of growth of the trifoliate leaf blades was used in the present experiments as a partial indication of the amount of translocation, the writer is fully aware that this is not an absolute measure and used it only because of the lack of a better method. MITCHELL *et al.* (10), after applying 2-iodo-3-nitrobenzoic acid containing radioactive iodine to one primary leaf of young bean plants, found that growth of the terminal buds was reduced roughly in proportion to the amount of the growth-regulator accumulated in them until a concentration of $2.7 \mu\text{g.}/50 \text{ mg.}$ of dry tissue was reached. At higher concentrations, no greater inhibition occurred. WEAVER (13) reported a strong inverse correlation between the weight of trifoliate leaf blades of the Red Kidney bean and the amount of 2,4-D earlier applied in aqueous sprays. In the present investigation a negative correlation was found between the amount of $\text{NH}_4(2,4\text{-D})$

D) absorbed in experiments A and B and the fresh weight of the harvested trifoliolate leaf blades for all plants, regardless of the time of clipping the treated primary leaves. In experiment B this correlation was highly significant (0.01 level) for all plants except those from which the treated leaves were clipped 4 hours after application. In experiment A the correlation was statistically significant for the plants from which the treated primary leaves were clipped after 24 and 48 hours but not for the others. The limited translocation which had occurred during the first 4 hours after application perhaps accounts for this lack of a significant level of correlation in those plants from which the treated primary leaves were then clipped.

There was no consistent statistical correlation between the amount of the salt absorbed and the degree of stem curvature at the time of removing the treated leaf. It was positive, however, for the 4- and 10-hour clippings in experiments A and B and significantly so at both times in experiment B. As previously pointed out, the greatest average curvature after the first 4-hour period consistently occurred in the intermediate temperature range, although more of the salt was absorbed in the highest temperature range. Apparently there are many factors which condition the amount of stem curvature resulting from a given treatment. Consequently, the degree of curvature definitely is not an accurate measure of the amount of a given growth substance which has entered a plant even though such use has been made of it in the past.

In conclusion, Carbowax 1500 apparently increases the effectiveness of various growth substances applied to the kidney-bean leaf in aqueous solution by causing enough moisture to be retained on the treated area to permit absorption

of the material over a longer period of time, thus effecting a greater amount of absorption of the growth-regulator. Carbowax does not appear to have any effect on the rate of translocation of such compounds, although the evidence is insufficient for a definite conclusion. Rate and amount of absorption of $\text{NH}_4(2,4\text{-D})$ by the leaves of kidney-bean plants are positively correlated with temperature. The evidence as to the effects of temperature on its translocation is not conclusive. Rate and amount of absorption of the salt are greater in the dark than in the light, but various light intensities (100 f.-c. or above) do not appear to have any effect on absorption if temperature is constant. There apparently is no translocation of $\text{NH}_4(2,4\text{-D})$ out of the treated leaf in the dark, or, if translocated, it is not effective in evoking responses in the remainder of the plant. The rate of translocation in the light, however, is apparently positively correlated with intensity, at least in the lower intensities. In general, these results substantiate the conclusions of the majority of other workers (7, 14) that translocation of the growth stimulus occurs most rapidly under conditions favoring photosynthesis in, and the movement of food materials from, the treated area.

Summary

1. Young Red Kidney bean plants were treated by applying a known amount of ammonium 2,4-dichlorophenoxyacetate [$\text{NH}_4(2,4\text{-D})$] to the base of the midrib on the upper surface of one primary leaf. The treated leaves were cut off at the bases of the petioles at various intervals (4, 10, 24, 48, and 72 hours) after application so as to interrupt further translocation of the growth-regulator from the leaf to other parts of the plant. Fresh weights of the expanded

trifoliolate leaf blades on treated and control plants 9 days after application of the salt were used in evaluating the relative amounts of growth inhibition which resulted for the various time intervals during which its translocation from the leaf was possible. The average degree of stem curvature at the time of clipping the treated primary leaves was also used as a supplementary indication of the amount of such translocation. To determine how much $\text{NH}_4(2,4\text{-D})$ was apparently absorbed by each leaf, the treated area was washed volumetrically with distilled water. The washings were tested for content of the salt by a slight modification of the method developed by BANDURSKI (1). The amount left on the surface of a treated leaf at any time and thus measured was subtracted from the amount applied to give an approximate measure of the amount absorbed.

2. Much of the absorption of $\text{NH}_4(2,4\text{-D})$ from a wholly aqueous solution by the leaf occurred within the first 4 hours after application. The addition of 0.5% by weight of Carbowax 1500 to the solution resulted in the salt being absorbed continuously during the period of treatment (72 hours).

3. The amount of the salt absorbed was positively correlated with temperature values during treatment, both with and without Carbowax.

4. There was no significant difference between the amounts of the salt absorbed at 100 foot-candles of light and at 900 foot-candles at the same air temperature; the amount absorbed in the dark was significantly greater.

5. The fresh weight of the trifoliolate leaf blades of plants treated in the dark was essentially the same as that of the corresponding controls, and no stem cur-

vature occurred. In addition, the treated primary leaves folded upward along the midrib and became wavy. These facts suggest that, though there may have been movement of $\text{NH}_4(2,4\text{-D})$ within the treated leaf, no translocation from the treated leaf occurred in complete darkness.

6. The relative fresh weights (expressed as a percentage of the average of the controls) of the trifoliolate leaf blades of plants treated at 100 foot-candles of light were considerably greater than those of plants treated at 900 foot-candles, even though the amounts of the growth-regulator absorbed were the same. The average degree of stem curvature was considerably higher in the latter series. Moreover, the primary leaves treated at the lower intensity tended to become wavy, similar to those in the dark but in lesser degree, while those at 900 foot-candles were not visibly affected. All these facts suggest that the rate of translocation of $\text{NH}_4(2,4\text{-D})$ from the leaf in the kidney bean is positively correlated with light intensity prevailing during treatment, at least for the lower intensities.

7. The available data do not indicate any effect of Carbowax 1500 on translocation of $\text{NH}_4(2,4\text{-D})$ from the treated leaf.

8. Data concerning the effects of temperature on the rate of translocation of the growth-regulator were inconclusive.

The writer acknowledges with sincere thanks the advice and assistance given by Dr. CHARLES E. OLMSTED, Mr. ROBERT S. BANDURSKI, and Dr. ROBERT J. WEAVER of the Department of Botany of the University of Chicago during the course of this investigation.

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